each tumour were processed for fluorescence microscopy according to the method of FALCK and HILLARP[8].

Histochemically, the tumour cells were found to emit a formaldehyde-induced green fluorescence which was confined to the cytoplasm. The intensity was moderate to strong. No other structures displayed formaldehyde-induced fluorescence. No difference between the tumour cells of NSD 1015 treated and non-treated animals could be seen in the fluorescence microscope. The observations completely agreed with those previously obtained[2]; thus, judged by the fluorescence microscopy, the continued transfers of the tumour had not brought about any changes in the cellular fluorigenic substances.

The recorded dopa-decarboxylase and MAO activities and the concentrations of dopa and dopamine in the tumours are summerized in the Table. The concentrations of dopa and dopamine found in this study agree well with those earlier reported[2].

The concomitant occurrence of dopa and a relatively high dopa-decarboxylase activity strongly suggests that the dopamine stored in the tumour cells is formed within them. In fact, the rate of formation may well be high, since inhibition of the dopa-decarbocylase resulted in a dramatic increase of dopa in the tumour as was evident from both the fluorimetric and paper chromatographic analyses. The finding of high amounts of dopamine after dopa-decarboxylase inhibition need not be contradictory to this view. As can be seen from the Table a complete inhibition was not obtained with NSD 1015, and the remaining enzyme activity in combination with the highly increased amounts of the substrate may well account for a maintenance of the dopamine level. It should be noted that the inhibition of dopa-decarboxylase may have been more effective in vivo than the recorded figure denotes owing to sources of error in the determination procedure. Thus, e.g. the dilution of the homogenized tissue in the incubation mixture and the presence of excessive amounts of pyridoxal-5-phosphate may to a certain extent reverse the effect of NSD 1015.

As 5-hydroxytryptophan in other tissues is also a good substrate[9,10] for dopa-decarboxylase, the 5-hydroxytryptamine that has recently been demonstrated to occur in the tumour[2] may also be synthesized by the tumour cells. However, it remains to be evaluated whether the second pre-requisite for the monoamine synthesis, the hydroxylating enzyme, is also present in the tumour.

The estimated activity of the MAO seems to be consistent with the idea that the tumour cells possess the ability for oxidative deamination of their monoamines in agreement with recent findings by GRILLO[11].

The presence of the 2 enzymes is a feature which the insulin-producing tumour cells and normal endocrine pancreatic cells share in common. Thus, evidence was produced that exogenous L-dopa is taken up into islet cells of the mouse and subsequently decarboxylated to dopamine, which in turn is exposed to MAO within the cells[3]. MAO in islet cells of many mammalian species has also been demonstrated by means of the tetrazolium method[12].

Zusammenfassung. In einem transplantierbaren Inselzelltumor des Goldhamsters, der neben 5-Hydroxytryptamin, Dopa und Dopamin eine bisher unidentifizierte, vermutlich monoaminähnliche Substanz enthält, wurde eine hohe Dopadecarboxylase- und Monoaminooxydase-aktivität gefunden. Nach Dopa-decarboxylasehemmung wurde eine starke Anhäufung von Dopa im Tumorgewebe beobachtet; dieser Befund weist auf eine schnelle Monoaminsynthese und einen schnellen Monoaminumsatz im Tumorgewebe hin.

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The Growth of Allogeneic Tumour Cells in Wasting, Cyclophosphamide-Treated Rats

We have previously found that rats, treated with a single dose of 110 mg cyclophosphamide/kg body weight, after the initial weight loss during the first 2–4 days and a subsequent, apparently normal weight gain, began to lose weight again and died in a wasting disease after one to several months[1,2]. In the terminal stage, the rats showed the impaired hair growth, hunched posture and high-stepping gait, described as characteristic of runting. It was considered to be of interest to study the cyclophosphamide-treated, wasting rats in greater detail. As a part of such an investigation, the present paper will give a brief report on the growth of allogeneic tumour cells in cyclophosphamide-treated rats.

Experiments. One hundred 3-week-old, male, white, non-inbred rats of the Sprague-Dawley strain (AB Anticimex, Stockholm, Sweden) were given a s.c. injection of either 110 mg cyclophosphamide ('Sendoxan', kindly supplied by AB Pharmacia, Sweden) per kilogram body weight, or the equivalent amount of distilled water. On the following day, or on the 55th day after these treatments, when the cyclophosphamide-treated rats were entering the wasting stage (see the Figure), the rats were injected s.c. into the back near the head with viable tumour cells in the numbers denoted in the Table. The tumour cells were from the in vitro tumour cell line, previously used and described[3], obtained from an SV 40 rat sarcoma[4]. The in vitro passages employed were 348 and 371

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Tumour take in cyclophosphamide-treated rats

Treatment	Time between treatment and cell inoculation	Number of cells inoculated 10	10^2	10^3	10^4	10^5	10^6
Cyclophosphamide	24 h	3/3/5	5/5/5	5/5/5	3/3/3	3/3/3	not done
Water	24 h	2/2/5	1/1/5	3/3/5	3/3/4	5/5/5	not done
Cyclophosphamide	55 days	1/1/2	0/0/4	1/0/4	0/0/3	1/1/4	4/0/4
Water	55 days	0/0/4	0/0/4	0/0/4	0/0/4	1/0/4	1/1/4

Table gives total number of tumours over number of lethal tumours over number of animals. Some rats that died soon after the treatment with cyclophosphamide or cell inoculation are excluded from the table.

for day one and day 55, respectively. Tumour growth and body weight were followed. Rats with tumours larger than 5 cm in diameter were killed, because, according to our experience, tumours of this size do not regress, and we wanted to spare the rats from unnecessary suffering. All tumours were examined histologically and found to be fibrosarcomas of the type previously described[3]. The rats were fed on rat pellets and tap water ad libitum.

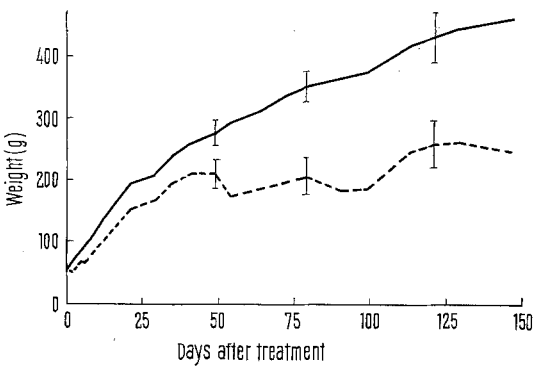
From the 55th day until the end of the experiment all rats were kept in a special room under scrupulous cleanliness to permit as long survival as possible of the cyclophosphamide-treated rats so that possible tumours should have time to grow out before the death of the animals.

Results and discussion. In the present experiments, the rats were treated with cyclophosphamide at 3 weeks of age, a time at which rats are not fully mature from an immunological point of view, and inoculated with tumour cells either 24 h or 55 days later. At the latter time the cyclophosphamide-treated rats were entering the 'wasting' stage, as indicated by the growth curves of the animals (see the Figure) and the fact that some of the animals, not included in the Figure or Table, died. By careful treatment of the remaining rats, most of them survived, however, for several weeks.

The Table shows the total number of tumours that developed and the number of lethal tumours. There was a high tumour take in the 3-week-old control rats in agreement with our previous observations[1,3]. There was a still higher tumour take in the 3-week-old rats, treated with cyclophosphamide 24 h earlier. That indicates a decreased immunological response in these rats, which agrees with the known immunosuppressive effect of the drug[5–9]. The result also agrees with the finding of increased growth of allogeneic tumour cells in mice submitted to immunosuppression by cortisone or antilymphocyte serum[10]. It was, however, not examined whether a possible difference in food intake between cyclophosphamide-treated and control rats might have been of importance for the results.

The control rats, inoculated with tumour cells at 11 weeks of age, showed a very low tumour take, arguing for a considerably increased immunological response against the tumour cells at this age as compared to 3 weeks of age. The rats, inoculated with tumour cells at 11 weeks of age and treated with cyclophosphamide at 3 weeks of age, showed a slightly higher tumour take than the 11-week-old controls. They showed, however, a considerably lower tumour take than the rats inoculated with tumour cells at 3 weeks of age, whether the latter had been treated with cyclophosphamide or not. This indicates that treatment with cyclophosphamide at 3 weeks of age did not prevent the development of considerable immunological response in the respect studied, though the rats were entering the 'wasting' stage[11].

Zusammenfassung. Cyclophosphamid bewirkt in jungen Ratten 1. eine Schwächung der Immunabwehr allogener Tumorzellen, 2. «wasting disease». Die beiden Wirkungen gehen nicht parallel: die «wasting disease» entwickelt sich einen bis mehrere Monate nach Cyclophosphamid-Gabe, zu einem Zeitpunkt, da sich das Immunsystem weitgehend wieder erholt hat.



The mean weight of the 2 groups of rats, inoculated with tumour cells on the 55th day after the treatment. The rats that died of tumour or wasting are included in the Figure. The standard deviations of the groups are shown for days 49, 79 and 121. The differences between the groups on these days were statistically highly significant ($P < 0.001$). ---, treated with cyclophosphamide; —, controls.

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